SIMPLIFIED PROCEDURE FOR CRYPRESERVING CELLS IN CRYOVIALS

This procedure uses simplified methods for cryopreserving vials without the use of the Cryomed controlled rate freezer. **STERILE TECHNIQUE SHALL BE USED IN THIS PROCEDURE**

1. Determine the number of cells to be frozen down in each cryovial. For 1 ml total volume cryovials there needs to be 4-50 million cells in each vial. For ½ ml total volume cryovials there needs to be 1-3 million cells in each vial.
2. Determine the number of cryovials to be frozen down.
3. Create the correct amount of freeze solution based on the number of vials to be cryopreserved. This solution needs to be chilled prior to use. (refer to the freeze solution procedure for preparation) For 1 ml cryovials there will need to be ½ ml cell suspension and ½ ml freeze solution added. The exception to this is for 1.5 million cell aliquots. They will require ¼ ml cell suspension and ¼ ml freeze solution.
4. Label all the cryovials with the appropriate information needed.
5. When all are labeled the cryovials can be placed on a rack. The rack of vials can then be placed in the biological safety cabinet. Now is best time to loosen the tops of the cryovials. Do not remove the tops from the vials at this point in time.
6. Depending on the cell concentration the volume of the cell suspension will need to be increased or decreased. If the volume should need to be increased use sterile Miltenyi buffer. If the volume needs to be decreased, then centrifuge the cells in the Sorvall tabletop centrifuge for 10 minutes at 1600 RPM with brake at room temperature. When the centrifugation is complete, remove an adequate volume of supernatant to have the final correct cryopreservation volume determined from steps 2 and 3.
7. Make sure the cells are well mixed once the final volume has been reached.
8. Pipet ½ ml of the well mixed cells into each of the cryovials. (for 1 ml total volume cryovials)
9. When completed add ½ ml of chilled freeze solution to each of the cryovials. (for 1 ml total volume cryovials)
10. When all the cells and freeze solution has been added to all the cryovials make sure all the caps are tightly screwed down. Invert the rack holding vials to ensure the freeze solution and cell suspension are well mixed. This can be done a couple of times. After the last time tap the base of the rack containing the vials with the surface of the biological safety cabinet. This will force down any liquid that is caught in the cryovial caps. (go to step 15)
11. If you are making 1.2 million cell aliquots, then the total volume of the cells and freeze solution will be ½ ml.
12. It is best to combine the freeze solution to the cell suspension prior to distribution into the cryovials. Make sure the cell suspension and freeze solution are well mixed prior to pipetting this mixture into the cryovials.
13. Once all the cells/freeze solution mixture is pipetted tightly cap the cryovials.
14. DO NOT INVERT THE VIALS!

15. The vials can now be placed in a Styrofoam rack. When all the vials are in the rack place the Styrofoam rack into a larger Styrofoam box. This box can then be placed in a -80 C freezer for at least 3 hours.
16. After at least 3 hours, the vials can be removed and placed in the vapor phase of a liquid nitrogen freezer for long term storage.

MATERIALS REQUIRED FOR THIS PROCEDURE

- Cryovials
- Pipets
- Freeze solution
- Miltenyi buffer
- Vial rack
- Styrofoam vial rack
- Styrofoam box
- -80 C freezer
- Sorvall tabletop centrifuge
- Falcon tubes